investigate effect of various system parameters, such as ligand density and mechanical properties of the receptor-ligand interaction, on the kinetics and mechanics of cell adhesion process. More importantly, this study provides a computational framework, with multi-scales and multi-physics, that can be extended for better controlling of cell interactions at the cell-biomaterial interface and for modeling the cell motility.

2972-Pos
A Search for Energy Minimized Sequences of Proteins
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Computational design of sequences for a given structure is generally studied by exhaustively enumerating the sequence space, which is prohibitively expensive. However, we point out that the protein topology has a wealth of information, which can be exploited to design sequences for a chosen structure. We design a computationally efficient method for ranking the residue sites in a given native-state structure, which enables us to design sequences for a chosen structure. The premise for the method is that the topology of the graph representing the energetically interacting neighbors in a protein plays an important role in the inverse-folding problem. We use edge-weighted connectivity graph for ranking the residue sites with reduced amino acid alphabet and then use continuous optimization to obtain the energy-minimizing sequences. Our methods enable the computation of a lower bound as well as a tight upper bound for the energy of a given conformation. We validate our results by using three different inter-residue energy matrices for five proteins from protein data bank (PDB), and by comparing our energy-minimizing sequences with 80 million diverse sequences that are generated based on different considerations in each case. Some of our chosen energy-minimizing sequences are similar to the sequences from non-redundant protein sequence database with an E-value of the order of 10^{-7}. In summary, we conclude that proteins show a trend towards minimizing energy in the sequence space but do not seem to adopt the global energy-minimizing sequence. The reason for this could be either that the existing energy matrices are not able to accurately represent the inter-residue interactions in the context of the protein environment or that Nature does not push the optimization in the sequence space, once it is able to perform the function.

2973-Pos
Simplified Theory for DNA Melting Maps
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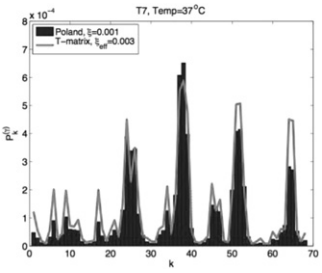
DNA melting maps on DNA stretched on surfaces or in nanochannels give a coarse grained picture of the underlying sequence with potential applications in studies of structural variations and for identification of (micro)organisms. The underlying mechanism is based on the difference in free energies associated with breaking AT and GC basepairs so that DNA melts first in AT-rich regions and only at higher temperature in GC-rich regions. With a suitable choice of dye the melted regions and the unmelted regions can readily be distinguished.
The Poland-Scheraga (PS)model is an Ising model with a long-range term due to the entropy associated with the single-stranded regions and, although computationally slow (~ square of the number of basepairs), has proven to well reproduce melting data. However, by adapting our algorithms to the resolution of the experimental melting mapping (1kbp) we can make them computationally more efficient.
We combine a transfer matrix approach and an exact Poland-type algorithm to study opening probabilities along DNA. We systematically explore different degrees of simplifications such as capping the long-range interactions or using a coarse-grained effective-medium approach. We evaluate our simplifications against exact solutions to the PS model for known sequences (figure).



2974-Pos
Testing a Hybrid Solvation Model with a Transition Layer Via Molecular Dynamics Simulation
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We previously developed a three dielectric layer hybrid solvation model for the electrostatic interactions of biomolecules in solvents using the linearized Poisson-Boltzmann equation. In this model, the interior spherical cavity contains the solute and explicit solvent molecules. Rather than employing the commonly invoked classical Kirkwood model that assumes a discontinuous change in dielectric constant from inside to outside the sphere, we introduced an intermediate buffer layer. Outside the spherical shell defines the exterior layer, where bulk solvent is modeled implicitly and characterized by a dielectric constant. Within the buffer layer, a special dielectric permittivity profile is constructed to give a continuous transition from the interior cavity to the exterior region. The purpose of the buffer layer is to remove unphysical divergence in electrostatic force at the cavity boundary. The electrostatic force within the cavity due to the reaction field of solvents with various ionic strengths is calculated using discrete image charges. Molecular dynamics simulation is performed using a recently developed simulation protocol to benchmark the effectiveness of the buffer layer, for various thickness, h, and different ionic concentration. Monitoring response functions and distributions of force and torque on molecular water facilitates relative comparisons. This work is supported by NIH 1R01 GM083600-03.

2975-Pos
Data-Driven Analysis of Cell Motility on Nanostructured Surfaces
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Experimental time series for trajectories of motile cells contains so much information that a systematic analysis yields cell-type-specific motility models. Using a range of cell types on various nanostructured surfaces we have explored how the surface type and cell type result in different motility models. This reflects the cells' different roles in the organism by showing that a cell has a memory of past velocities. They also suggest how the nanopatterns imprinted on the various surfaces affect cell motility.

2976-Pos
Development and Application of Non-Additive Force Fields for Molecular Simulations of Lipid Bilayers and Integral Membrane Proteins
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Molecular simulations today are applied across many scientific disciplines. Complementing experiment, these tools afford a molecular-level understanding and interpretation of physico-chemical processes at resolutions and timescales difficult or practically inaccessible to experiment. At the heart of such methods is the description of interactions between atoms and molecules, the force field. Traditionally, non-reactive force fields have treated electrostatic interactions using an additive, Coulomb model between fixed partial charges on atomic sites. Though quite successful, there has been conjecture as to the effects of incorporating non-additivity in classical force fields, particularly in biological systems. Over the last several decades, attempts to incorporate electrostatic non-additivity in the form of inducible dipole interactions or dynamically varying partial charges have provided a vast body of knowledge that has aided in the development of a new class of force fields attempting to explicitly account for non-additive effects. We will present our recent work in developing one such class of models, charge equilibration force fields, and applications of such models to aqueous solution interfaces, membrane bilayers and simple integral membrane peptides such as the gramicidin A bacterial channel, and recent work on modeling of protein-ligand interaction free energetics.

Imaging & Optical Microscopy III

2977-Pos
In Situ Measurements of Oligomerization State of NBCe1-A in Rat Kidneys Via Spatial Fluorescence Intensity Fluctuation Analysis
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NBCe1-A plays an important role in absorbing sodium bicarbonate across the basolateral membrane of the proximal tubule. We have previously showed that minimal functional unit of NBCe1-A is a monomer, and based on in-vitro biochemical studies in HEK293 cells, the oligomeric state of the cotransporter was shown to be predominantly dimeric with monomeric and higher oligomeric forms also present. We developed an in situ measurement methodology to determine the oligomeric state of NBCe1-A without requiring tissue disruption

using biochemical methods. We used fluorescent moment image analysis and spatial intensity distribution analysis (SpIDA) to study the oligomeric state of NBCe1-A in cultured cells expressing the cotransporter and in rat kidney tissue. Both methods allow for quantitative measurement of fluorescent particle densities and oligomerization states within individual images acquired with laser-scanning microscopy. Initially we examined basal membranes of highly adherent CHO K1 cells expressing eGFP-tagged NBCe1-A because of their large surface area. As an independent control of monomeric brightness, we used cells expressing monomeric eGFP anchored to the membrane. Taking into account the recovered values of the monomeric eGFP quantal brightness, we show that NBCe1-A exists in monomeric and dimeric states on the cell membrane. We also used an Alexa488-alpha-bungarotoxin conjugate to label cells expressing an NBCe1A-bungarotoxin binding mutant. As a monomeric control, we immobilized Alexa488 dye on cover slips. The spatial fluorescence intensity fluctuation analysis revealed a similar distribution of aggregates as shown for eGFP data. Moreover, we immunolabeled NBCe1-A in rat kidney tissues as well as in cultured HEK293 cells expressing the cotransporter demonstrating the NBCe1-A is present in monomeric, dimeric and rarely in higher order oligomeric states. These experiments demonstrate for the first time the in situ oligomeric state(s) of NBCe1-A.

2978-Pos

Assessing the Mutagenicity Potential of Multiphoton Excitation during Imaging of Intrinsic Fluorescence from Cells and Tissues

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Multiphoton-excited intrinsic fluorescence emission signals from cells and tissues can be used for metabolic imaging and studies of disease morphology in chronic animal imaging experiments, and has diagnostic potential as a future in vivo clinical imaging tool. However, the signals are generally weak and require high laser intensities for imaging. Consequently, not only are viability studies important, but an investigation into the extent that multiphoton excitation is a mutagenic agent is critical. Information on the magnitude of permissible intensity levels of femtosecond pulsed near-IR light is vital to human clinical applications, yet there are currently no regulations that specifically indicate such parameters. This study is directed towards determining whether multiphoton imaging of cellular autofluorescence using 700 - 800 nm wavelength excitations causes mutations in mammalian cells. The induction of mutation by pulsed laser radiation employed for multiphoton imaging entails a risk of carcinogenicity in living tissue. The assessment of potential laser illumination toxicity was carried out by the hypoxanthine-guanine phosphoribosyl (HPRT) mammalian cell gene mutation assay, which measures mutation at the HPRT gene locus in cells, and is one of a handful of reporter loci that have been used as molecular biomarkers for both human and rodent exposure to mutagens and UV light. Experiments were performed to assess possible mutagenic effects of various intensities of 755 nm, 100 fs laser irradiation. Laser powers ranging from 20 to as high as 100 mW delivered as raster scanned excitation through a 0.7 NA objective for 20 seconds was found to be nonmutagenic to the HPRT gene locus test system, while higher laser powers initiated mutagenic responses.

2979-Pos

Optical Analysis of Calcium Channels at the First Auditory Synapse

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Transmitter release at the first auditory synapse, the ribbon synapse of cochlear inner hair cells (IHCs), is tightly regulated by Ca^{2+}. Using fast confocal Ca^{2+} imaging, we have recently described pronounced differences in presynaptic Ca^{2+} signals between single synapses within the same cell. These Ca^{2+} microdomains differed both in their amplitude and voltage-dependence of activation.

As for the mechanism behind the amplitude heterogeneity, we provided indirect evidence for differences in the Ca^{2+} channel complement, pointing towards a differential regulation of Ca^{2+} channel number (N_{Ca}) across synapses. Moreover, a very simplistic model reveals potential consequences of different Ca^{2+} channel complements for sound encoding at different synapses

In order to directly study synaptic Ca^{2+} channels, we are currently implementing an optical fluctuation analysis approach. Here, we present preliminary results along potential caveats. This work provides a framework of how to further dissect presynaptic Ca^{2+} microdomain heterogeneity - likely being involved in determining the diverse responses of the postsynaptic neurons, which, as a population, encode the huge range of perceived stimulus intensities (sound pressure varying over 6 orders of magnitude).

2980-Pos

Reactive Oxygen Species as Essential Mediators of Cell Adhesion and Migration

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In recent years reactive oxygen species (ROS) and by extension changes in the intracellular reductive/oxidative (redox) balance have come into focus as major regulators of key cellular functions in both physiological and pathological settings. Traditionally viewed as mediators of cell damage by exogenous noxae, oxygen intermediates have been also recognized of signaling roles downstream of cytokine and mitogen receptors, activated oncogenes, nutrient sensors and pro-apoptotic stimuli, when endogenously generated by a number of intracellular biochemical sources. The signaling properties of ROS are largely due to the reversible oxidation of redox-sensitive target proteins, and especially of protein tyrosine phosphatases, whose activity is dependent on the redox state of a low pKa active site cysteine. Cell spreading, adhesion and migration requires ROS generation and interaction with protein tyrosine phosphatases downstream of adhesion molecules. We have taken advantage of a redox-sensitive mutant of the Yellow Fluorescent protein (rxYFP), employed ratiometrically, to draw real-time redox maps of adhering and migrating cells. A quantitative analysis of redox maps allows to disclose a peculiar spatial organization of the redox environment, providing evidence that intracellular ROS are generated after integrin engagement and that these oxidant intermediates are necessary for integrin signaling during cell spreading, adhesion and migration. Taken together these observation support the application of rxYFP in the subcellular mapping of physiological dynamic redox phenomena involved in signal transduction.

2981-Pos

Real Time Imaging of Endogenous MRNAs during Stress

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During stress, cytoplasmic mRNAs aggregate to RNA granules such as stress granule (SG) and processing body (PB), where they are sorted and remodeled for reinitiation of translation, degradation or storage. Till now, stress-induced assembly of these granules is mainly studied indirectly by using protein markers, and the real time behavior of endogenous mRNAs in living cells has not been detected directly yet and remained uncertain.

Here we used a linear antisense probe to visualize endogenous cytoplasmic mRNAs in living mammalian cells in order to investigate the dynamics of mRNAs under stress. A Cy3 and biotin labeled poly$(U)_{22}$ 2'-*O*-methyl RNA probe was prepared for the detection of poly$(A)^+$ mRNAs. The probe combined with streptavidin was microinjected into the cytoplasm of COS-7 cells, followed by the inducement of 0.5 mM arsenite stress. We also transfected TIA-1-GFP plasmid into the cells by FuGENE 6 to determine SG. As a result, mRNAs visualized by the antisense probe aggregated to granules during stress and the granules colocalized with SG marked by TIA-1-GFP. Next, the number and size of the granules were studied by real time imaging. mRNAs rapidly aggregated to form clusters within 20 min in response to stress. A large amount of small granules first emerged, gradually gathered to bigger ones about 30 min after the inducement of stress.

In this study, the aggregation of endogenous mRNAs to SG was successfully visualized by using the linear antisense probe. The behavior of endogenous mRNAs in SG will be revealed.

2982-Pos

Imaging Dopamine and Serotonin in Live Neurons with Multi-Photon Excited Ultraviolet Auto-Fluorescence

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Monoamine neurotransmitters are implicated in mood, aggression, reward, and addiction pathways in the mammalian brain. However, visualizing them in live neurons with sub-micron resolution has remained a challenge. It is difficult to label them fluorescently, and their intrinsic ultraviolet fluorescence is difficult to access. Unlike serotonin which can be imaged with three-photon microscopy, dopamine presents a special challenge due to its shorter wavelength (~300nm) emission. We now show that dopamine can be imaged with sub-micron resolution in live brain slices with a combination of a non-epifluorescent collection design, special optical elements, and two-photon excitation with a visible femtosecond laser. Substantia Nigra (SN) tissue sections from the